A P P L I C A T I O N

for

UNITED STATES LETTERS PATENT

on

METHOD OF IDENTIFYING MOLECULES THAT HOME TO A SELECTED ORGAN *IN VIVO*

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Sheets of Drawings: None

Docket No.:   P-LJ 4859

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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METHOD OF IDENTIFYING MOLECULES THAT HOME
TO A SELECTED ORGAN *IN VIVO*

This application is a continuation of application U.S. Serial No. 08/862,855, filed

5 May 23, 1997, which is a continuation-in-part of U.S. Serial No. 08/813,273 filed March 10, 1997, which is a continuation-in-part of U.S. Serial No. 08/526,710, filed September 11, 1995, each of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

15 ### FIELD OF THE INVENTION

The present invention relates generally to the fields of molecular medicine and drug delivery and, more specifically, to a method of *in vivo* panning for identifying a molecule that homes to a specific organ.

20 ### BACKGROUND INFORMATION

Although the effect of a particular pathology often is manifest throughout the body of the afflicted person, generally, the underlying pathology may affect only a single organ or tissue. In many cases, drugs are

25 the treatment of choice for a patient suffering a particular disease. It is rare, however, that a drug will target only the diseased tissue or organ. More commonly, drug treatment results in undesirable side effects due, for example, to generalized toxic effects

30 throughout the patient's body. The nausea, loss of hair and drop in blood count that occur as a result of treating of a cancer patient with chemotherapeutic agents

are examples of the undesirable side effects that can occur due to drug treatment.

The undesirable side effects that can occur when drugs are used to treat a disease most often are due to the inability of the drug to specifically target the diseased organ or tissue. For example, a cancer chemotherapeutic agent that targets rapidly proliferating cells would be useful to kill rapidly dividing cancer cells. However, such an agent also kills normal proliferating hematopoietic and epithelial cells. Thus, the dose of such a drug that can be administered to a patient is limited due to its toxic effect on normal cells.

Efforts have been made to increase the target specificity of various drugs. In some cases, a particular cell type present in a diseased tissue or organ may express a unique cell surface marker. In such a case, an antibody can be raised against the unique cell surface marker and a drug can be linked to antibody. Upon administration of the drug/antibody complex to the patient, the binding of the antibody to the cell surface marker results in the delivery of a relatively high concentration of the drug to the diseased tissue or organ. Similar methods can be used where a particular cell type in the diseased organ expresses a unique cell surface receptor or a ligand for a particular receptor. In these cases, the drug can be linked to the specific ligand or to the receptor, respectively, thus providing a means to deliver a relatively high concentration of the drug to the diseased organ.

While linking a drug to a molecule that homes to a particular cell type present in a diseased organ or tissue provides significant advantages for treatment over the use of a drug, alone, use of this method is severely

limited.   In particular, very few cell type specific
antibodies have been described and it can be difficult
and time consuming to attempt to obtain an antibody that
targets an organ in a particular patient suffering a
pathology.   Furthermore, few cell type specific surface
markers have been described.   Even where such markers
have been described, the cells expressing the markers can
be distributed among various tissues or organs, thereby
limiting their usefulness as targets.   Thus, it is
important to identify specific target cell markers that
are expressed in only one or a few tissues or organs and
to identify molecules that specifically interact with
such markers.

Various cell types can express unique markers
and, therefore, provide potential targets for organ
homing molecules.   Endothelial cells, for example, which
line the internal surfaces of blood vessels, can have
distinct morphologies and biochemical markers in
different tissues.   The blood vessels of the lymphatic
system, for example, express various adhesion proteins
that serve to guide lymphocyte homing.   For example,
endothelial cells present in lymph nodes express a cell
surface marker that is a ligand for L-selectin and
endothelial cells in Peyer's patch venules express a
ligand for the $\alpha_4\beta_7$ integrin.   These ligands are involved
in specific lymphocyte homing to their respective
lymphoid organs.   Thus, linking a drug to L-selectin or
to the $\alpha_4\beta_7$ integrin may provide a means for targeting the
drug to diseased lymph nodes or Peyer's patches,
respectively, provided that these molecules do not bind
to similar ligands present in a significant number of
other organs.

Although the homing molecules present in the
blood vessels of non-lymphoid tissues have not been
clearly defined, the ability of lymphocytes to return to

the organ in which they were first stimulated indicates that organ-specific endothelial markers exist. Similarly, the homing or metastasis of particular types of tumor cells to specific organs provides further

5   evidence that organ-specific markers exist. However, there remains a need to identify other organ-specific cell markers and the molecules that bind to them.

Methods are now available for producing large populations of molecules. In addition, methods are
10 available for screening libraries of molecules to identify those of interest. For example, phage peptide display libraries can be used to express large numbers of peptides that can be screened *in vitro* with a particular target molecule or a cell of interest in order to
15 identify peptides that specifically bind the target molecule or the cell. Screening of such phage display libraries has been used, for example, to identify ligands that specifically bind various antibodies and cell surface receptors.

20 Screening of a phage display library generally involves *in vitro* panning of the library using a purified target molecule. Phage that bind the target molecule can be recovered, individual phage can be cloned and the peptide expressed by a cloned phage can be determined.
25 Such a peptide can be useful for delivery of a drug linked to the peptide to cells expressing the target molecule.

Unfortunately, very few target molecules that are expressed by only one or a few cell types have been
30 identified. Furthermore, even where such a target molecule is known, it is uncertain whether a peptide that specifically binds the target molecule, as determined using an *in vitro* panning method, will bind to the target molecule *in vivo*. As a result, the identification of a

peptide from a phage display library using an *in vitro* panning method essentially represents only a starting point for determining whether the identified peptide can be useful for an *in vivo* procedure. Thus, a need exists

5   to develop *in vivo* methods for screening large numbers of molecules such as peptides in order to identify those that can home to one or more selected organs. The present invention satisfies this need and provides related advantages as well.

10   **SUMMARY OF THE INVENTION**

The present invention provides a method of identifying a molecule that homes specifically to one or a few selected organs by using *in vivo* panning to screen a library of potential organ homing molecules. The *in*

15   *vivo* panning method involves administering a library, which consists of a diverse population of molecules, to a subject and identifying molecules that home to one or more selected organs in the subject. The invention is exemplified by administration of a phage peptide display

20   library to a subject and the identification of peptides expressed by phage that home to brain or to kidney in the subject. *In vivo* panning can be repeated one or more times until a molecule that homes to a selected organ with the desired selectivity is recovered.

25   The invention also provides methods of identifying a target molecule by detecting selective binding of the target molecule to a peptide of the invention. For example, a peptide that selectively homes to a selected organ can be attached to a solid matrix for

30   use in affinity chromatography. A sample of the organ can be obtained and passed over the affinity matrix under conditions that allow specific binding of the target molecule, which then can be collected and identified

using well known biochemical methods. The target
molecule can be useful, for example, for raising an
antibody specific for the target molecule.

DETAILED DESCRIPTION OF THE INVENTION

5   The present invention provides a method of
identifying a molecule that homes specifically to one or
a few selected organs by screening a library using *in
vivo* panning. The identified molecules are useful, for
example, for targeting a desired moiety such as a drug, a
10   toxin or a detectable label, which can be linked to the
molecule, to the selected organ. *In vivo* panning
provides a direct means for identifying molecules that
specifically home to a selected organ and, therefore,
provides a significant advantage over previous methods,
15   which require that a molecule identified using an *in
vitro* screening method subsequently be examined to
determine whether it maintains its specificity *in vivo*.

As used herein, the term "library" means a
collection of molecules. A library can contain a few or
20   a large number of different molecules, varying from about
ten molecules to several billion molecules or more. If
desired, a molecule can be linked to a tag, which can
facilitate recovery or identification of the molecule.

As used herein, the term "molecule" is used
25   broadly to mean an organic chemical such as a drug; a
peptide, including a variant or modified peptide or
peptide-like molecules such as a peptidomimetic or
peptoid; or a protein such as an antibody or a growth
factor receptor or a fragment thereof such as an Fv, Fd
30   or Fab fragment of an antibody, which contains a binding
domain. For convenience, the term "peptide" is used
broadly herein to mean peptides, proteins, fragments of

proteins, peptoids, peptidomimetics and the like. A molecule can be a non-naturally occurring molecule, which does not occur in nature, but is produced as a result of *in vitro* methods, or can be a naturally occurring

5 molecule such as a protein or fragment thereof expressed from a cDNA library.

Methods for preparing libraries containing diverse populations of various types of molecules are well known in the art and commercially available (see,

10 for example, Ecker and Crooke, Biotechnology 13:351-360 (1995), and Blondelle et al., Trends Anal. Chem. 14:83-92 (1995), and the references cited therein, each of which is incorporated herein by reference; see, also, Goodman and Ro, Peptidomimetics for Drug Design, in "Burger's

15 Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M.E. Wolff; John Wiley & Sons 1995), pages 803-861, and Gordon et al., J. Med. Chem. 37:1385-1401 (1994), each of which is incorporated herein by reference). Where a molecule is a peptide, protein or fragment thereof, the molecule

20 can be produced *in vitro* directly or can be expressed from a nucleic acid, which is produced *in vitro*. Methods of synthetic peptide and nucleic acid chemistry are well known in the art.

A library of molecules also can be produced,

25 for example, by constructing a cDNA expression library from mRNA collected from a cell, tissue, organ or organism of interest. Methods for producing such libraries are well known in the art (see, for example, Sambrook et al., Molecular Cloning: A laboratory manual

30 (Cold Spring Harbor Laboratory Press 1989), which is incorporated herein by reference). Preferably, the peptide encoded by the cDNA is expressed on the surface of a cell or a virus containing the cDNA. For example, cDNA can be cloned into a phage vector such as fuse 5

35 (see Example I), wherein, upon expression, the encoded

peptide is expressed as a fusion protein on the surface
of the phage.

As disclosed herein, *in vivo* panning comprises
administering a library to a subject, collecting a
5   selected organ and identifying an organ homing molecule.
An organ homing molecule can be identified using various
methods well known in the art. Generally, the presence
of an organ homing molecule in a collected organ is
identified based on one or more characteristics common to
10 the molecules present in the library, then the structure
of a particular organ homing molecule is identified. For
example, a highly sensitive detection method such as mass
spectrometry, either alone or in combination with a
method such as gas chromatography, can be used to
15 identify organ homing molecules in a selected organ.
Thus, where a library comprises diverse molecules based
generally on the structure of an organic molecule such as
a drug, an organ homing molecule can be identified by
determining the presence of a parent peak for the
20 particular molecule.

If desired, the selected organ can be processed
using a method such as HPLC, which can provide a fraction
enriched in molecules having a defined range of molecular
weights or polar or nonpolar characteristics or the like.
25 Conditions for HPLC will depend on the chemistry of the
particular molecule and are well known to those skilled
in the art. Similarly, methods for bulk removal of
potentially interfering cellular materials such as DNA,
RNA, proteins, lipids or carbohydrates are well known in
30 the art as are methods for enriching a fraction
containing an organic molecule using, for example,
methods of selective extraction. For example, where a
library comprises a population of diverse organic
chemical molecules each linked to a unique
35 oligonucleotide tag, such that the specific molecule is

identified by determining the oligonucleotide sequence
using PCR, genomic DNA can be removed from the sample of
the collected organ in order to reduce the potential for
background PCR reactions. These and other methods can be
5   useful for enriching the sample of the collected organ
for the particular organ homing molecule, thereby
removing potentially contaminating materials from the
collected organ sample and increasing the sensitivity of
detecting a molecule.

10   Evidence provided herein indicates that a
sufficient number of organ homing molecules selectively
home to a selected organ during *in vivo* panning such that
the molecules readily can be identified. For example,
various independent phage expressing the same peptide
15   were identified in brain and in kidney (see Tables 1
and 2). Specifically, almost half of the kidney homing
peptides that were sequenced had the amino acid sequence
CLPVASC (SEQ ID NO: 21). Similarly, two peptides that
homed to brain constituted about 40% of the sequenced
20   brain homing peptides. Thus, a substantial fraction of
the identified organ homing molecules can have the same
structure. Furthermore, the peptide inserts of only a
small number of isolated phage were determined. However,
following various *in vivo* pannings for brain and kidney
25   homing molecules, hundreds of thousands to millions of
phage expressing organ homing peptides were recovered
from the respective organs. These results indicate that
some organ homing molecules will be present in
substantial numbers in an organ following *in vivo* homing,
30   thereby increasing the ease with which the molecules can
be identified.

         Ease of identification of an organ homing
molecule depends on various factors, including the
presence of potentially contaminating background cellular
35   material. For example, where the organ homing molecule

is an untagged peptide, a larger number must home to the
organ in order to identify the specific peptides against
the background of cellular protein. In contrast, a much
smaller number of an untagged organic chemical homing

5   molecule such as a drug is identifiable because such
molecules normally are absent from or present in only
small numbers in the body. In such a case, a highly
sensitive method such as mass spectrometry can be used to
identify an organ homing molecule. The skilled artisan

10 will recognize that the method of identifying a molecule
will depend, in part, on the chemistry of the particular
molecule.

The molecules of a library also can be present
as a conjugate, which can facilitate recovery or

15 identification of the molecule. As used herein, the term
"conjugate" or "molecule conjugate" means a molecule of
the library linked to a physical, chemical or biological
tag such as a plastic microbead, an oligonucleotide or a
bacteriophage, respectively. The tag can provide a means

20 to identify or recover an organ homing molecule of the
invention following *in vivo* panning. For example, a
conjugate can be a molecule such as a peptide linked to a
unique oligonucleotide tag (see, for example, Brenner and
Lerner, <u>Proc. Natl. Acad. Sci., USA</u> 89:5381-5383 (1992),

25 which is incorporated herein by reference). Upon homing
to an organ, the particular peptide can be identified by
performing PCR on a sample of the organ containing the
conjugate under conditions that allow amplification of
the oligonucleotide tag (see, for example, Erlich, <u>PCR</u>

30 <u>Technology: Principles and Applications for DNA</u>
<u>Amplification</u> (Stockton Press 1989), which is
incorporated herein by reference). By determining the
oligonucleotide sequence, the identity of the peptide can
be determined.

In addition, a tag can be a support. As used herein, the term "support" means a tag having a defined surface to which a molecule can be attached. For example, a support can be a biological tag such as a

5   virus or virus-like particle such as a bacteriophage ("phage"); a bacterium such as *E. coli*; or a eukaryotic cell such as a yeast, insect or mammalian cell; or can be a physical tag such as a liposome or a microbead, which can be composed of a plastic, agarose, gelatin or other

10   material. In general, a support should have a diameter less than about 10 μm to about 50 μm in its shortest dimension, such that the support can pass relatively unhindered through the capillary beds present in the subject and not occlude circulation. In addition, a

15   support can be nontoxic and biodegradable, particularly where the subject used for *in vivo* panning is not sacrificed to collect a selected organ.

Where a molecule is linked to a support, the conjugate comprises the molecule attached to the surface

20   of the support, such that the part of the molecule suspected of being able to interact with a target in a cell in the subject is positioned so as be able to participate in the interaction. For example, where the molecule is suspected of being a β adrenergic agonist,

25   the binding portion of the molecule attached to a support is positioned so it can interact with a β adrenergic receptor on a cell in the selected organ. Similarly, where the molecule is suspected of being a ligand for a growth factor receptor, the molecule is positioned on the

30   support so that it can bind the receptor.

As exemplified herein, a peptide suspected of being able to home to a selected organ such as brain was expressed as the N-terminus of a fusion protein, wherein the C-terminus consisted of a phage coat protein. Upon

35   expression of the fusion protein, the C-terminal coat

protein linked the fusion protein to the surface of a phage such that the N-terminal peptide was in a position to interact with a target molecule in an organ. Thus, a conjugate was formed by the linking of a peptide molecule

5 to a phage, wherein the phage provided a biological support and the peptide molecule is linked as a fusion protein.

As used herein, the term "*in vivo* panning" means a method of screening a library by administering

10 the library to a subject and identifying a molecule that selectively homes to one or a few selected organs. The term "administering to a subject", when used in referring to a library of molecules or a portion thereof, is used in its broadest sense to mean that the library is

15 delivered to a selected organ. For example, a library can be administered to a subject by injecting the library into the circulation of the subject such that the molecules can pass through the selected organ; after an appropriate period of time, circulation is terminated by

20 sacrificing the subject or by removing a sample of the organ (see Example I). Alternatively, a cannula can be inserted into a blood vessel in the subject, such that the library is administered by perfusion for an appropriate period of time, after which the library can

25 be removed from the circulation through the cannula or the subject can be sacrificed or the organ can be sampled to terminate circulation. Similarly, a library can be shunted through one or a few organs by cannulation of the appropriate blood vessels in the subject. It is

30 recognized that a library also can be administered to an isolated perfused organ. Such panning in an isolated perfused organ can be useful for identifying molecules that bind to the organ and, if desired, can be used as an initial screening of a library. For example, if a kidney

35 homing molecule is desired, a library can be perfused through an isolated kidney, then the molecules that bound

to the perfused kidney can be screened by *in vivo* panning
to identify a kidney homing molecule.

The *in vivo* panning method is exemplified
herein by screening a phage peptide display library in
5   mice and identifying specific peptides that selectively
home to brain or to kidney (see Examples I and II).
However, phage libraries that display protein receptor
molecules, including, for example, an antibody or an
antigen binding fragment of an antibody such an Fv, Fd or
10   Fab fragment; a hormone receptor such as a growth factor
receptor; or a cell adhesion receptor such as an integrin
or a selectin also can be used to practice the invention.
Variants of such molecules can be constructed using well
known methods such as random, site-directed or codon
15   based mutagenesis (see Huse, U.S. Patent No. 5,264,563,
issued November 23, 1993, which is incorporated herein by
reference). Thus, various types of phage display
libraries can be screened using the disclosed *in vivo*
panning method.

20   Phage display technology provides a means for
expressing a diverse population of random or selectively
randomized peptides. Various methods of phage display
and methods for producing diverse populations of peptides
are well known in the art. For example, Ladner et al.
25   (U.S. Patent No. 5,223,409, issued June 29, 1993, which
is incorporated herein by reference) describe methods for
preparing diverse populations of binding domains on the
surface of a phage. In particular, Ladner et al.
describe phage vectors useful for producing a phage
30   display library, as well as methods for selecting
potential binding domains and producing randomly or
selectively mutated binding domains.

Similarly, Smith and Scott (Meth. Enzymol.
217:228-257 (1993); see, also, Scott and Smith, Science

249: 386-390 (1990), each of which is incorporated herein by reference) describe methods of producing phage peptide display libraries, including vectors and methods of diversifying the population of peptides that are

5   expressed (see, also, Huse, WO 91/07141 and WO 91/07149, each of which is incorporated herein by reference; see, also, Example I). Phage display technology can be particularly powerful when used, for example, with a codon based mutagenesis method, which can be used to

10   produce random peptides or randomly or desirably biased peptides (Huse, U.S. Patent No. 5,264,563, *supra*, 1993). These or other well known methods can be used to produce a phage display library, which can be subjected to the *in vivo* panning method of the invention in order to identify

15   a peptide that homes to one or a few selected organs.

In addition, to screening a phage display library, *in vivo* panning can be used to screen various other types of libraries, including, for example, an RNA or cDNA library or a chemical library. If desired, the

20   organ homing molecule can be in the form of a conjugate, which can facilitate recovery of the molecule from a selected organ or identification of the molecule in the organ. For example, where the molecules of a tagged chemical library are screened, the tag can be a moiety

25   such as biotin, which can be linked directly to the molecule or can be linked to a support containing the molecules. Biotin provides a means to recover the molecule from a selected organ using an avidin or streptavidin affinity matrix. In addition, a molecule or

30   a support containing a molecule can be linked to a hapten such as 4-ethoxy-methylene-2-phenyl-2-oxazoline-5-one (phOx), which can be bound by an anti-phOx antibody linked to a magnetic bead as a means to recover the molecule. Methods for purifying biotin or phOx labeled

35   conjugates are known in the art and the materials for

performing these procedures are commercially available (eg., Invitrogen; La Jolla CA; and Promega Corp.; Madison WI). In the case where a phage library is screened, the phage can be recovered using methods as disclosed in

5   Example I.

In *vivo* panning provides a method for directly identifying molecules that can home to one or a few selected organs. As used herein, the term "home" or "selectively home" means that a particular molecule binds

10   relatively specifically to a target molecule present in one or few selected organs following administration to a subject. In general, selective homing is characterized, in part, by detecting at least a 2-fold (2x) greater specific binding of the molecule to the selected organ as

15   compared to a control organ. It should be recognized that, in some cases, a molecule can localize non-specifically to an organ. Non-specific localization can be distinguished from homing by performing competition experiments such as those described in

20   Examples II.C. and II.D.

Selective homing can be demonstrated by determining the specificity of an organ homing molecule for the selected organ as compared to a control organ. For example, a ratio of brain:kidney homing of up to 9:1

25   was observed for brain homing peptides (i.e., 9x greater binding to the selected organ as compared to the control organ; see Example II).

Selective homing also can be demonstrated by showing that molecules that home to a selected organ, as

30   identified by one round of *in vivo* panning, are enriched for organ homing molecules in a subsequent round of *in vivo* panning. For example, phage expressing peptides that selectively home to brain were isolated by *in vivo*

panning, then were subjected to additional rounds of *in vivo* panning. As demonstrated in Example II, phage recovered from brain after a first round of screening showed an 8x enrichment in homing to brain as compared to

5 kidney following a second round of screening and a 13x enrichment following a third round of *in vivo* panning. When a peptide that selectively homes to brain was linked to a moiety, i.e., a red blood cell (RBC), the peptide/RBC complex selectively homed to brain (see

10 Example II.D.).

Due to the conserved nature of cellular receptors and of ligands that bind a particular receptor, the skilled artisan would recognize that an organ homing molecule identified using, for example, *in vivo* panning

15 in a mouse also would bind to the corresponding target molecule in the selected organ of a human or other species. For example, an RGD-containing peptide that can specifically bind to an integrin expressed by a cell in a human subject also can bind integrins expressed in a

20 variety of species, including integrins expressed in mammalian cells such as murine and bovine cells as well as in cells of more evolutionarily distant species such as *Drosophila*. The ability of an organ homing molecule identified using *in vivo* panning in an experimental

25 animal such as a mouse readily can be examined for the ability to bind to the corresponding organ in a human subject by demonstrating, for example, that the molecule also can bind specifically *in vitro* to a sample of the selected organ obtained from a human subject. Thus,

30 routine methods can be used to confirm that an organ homing molecule identified using *in vivo* panning in an experimental animal also can bind an organ-specific target molecule in a human subject.

The steps of administering the library to the subject, collecting a selected organ and identifying the molecules that home to the selected organ, comprise a single round of *in vivo* panning. Although not required,

5   one or more additional rounds of *in vivo* panning generally are performed. Where an additional round of *in vivo* panning is performed, the molecules recovered from the selected organ in the previous round are administered to a subject, which can be the same subject used in the

10   previous round, where only a part of the selected organ was collected.

By performing a second round of *in vivo* panning, the relative binding selectivity of the molecule conjugates recovered from the first round can be

15   determined by administering the identified molecule conjugates to a subject, collecting the selected organ and a control organ, and comparing the molecules recovered from the selected organ with those recovered from a control organ. In addition, a second round of *in*

20   *vivo* panning can indicate whether the molecules identified from the initially selected organ also can home to additional organs, thus defining a family of selected organs. Additional rounds of panning can be performed as desired.

25   Ideally, no molecules are recovered from a control organ following a second or subsequent round of *in vivo* panning. Generally, however, a proportion of the molecules also will be present in a control organ. In this case, the ratio of molecules in the selected organ

30   as compared to the control organ (selected:control) can be determined. As described above, for example, phage that homed to brain following a first round of *in vivo* panning demonstrated a 13x enrichment in homing to the

selected organ as compared to the control organ, kidney, following two additional rounds of panning (Example II).

Additional rounds of *in vivo* panning can be used to determine whether a particular molecule homes only to the selected organ or can recognize a target on the selected organ that also is expressed in one or more other organs or is sufficiently similar to the target in the originally selected organ. Where a molecule is found to direct homing to organs in addition to the originally selected organ, the organs are considered to constitute a family of selected organs. Using the method of *in vivo* panning, molecules that home to only a single selected organ and molecules that home to a family of selected organs can be defined. Such identification is expedited by collecting various organs during subsequent rounds of *in vivo* panning.

As used herein, the term "selected organ" is used in its broadest sense to mean an organ to which a molecule selectively homes. In addition, the term "organ" is used broadly to mean organ, tissue or cell type, including a cancer cell, in which case the selected organ can be a tumor such as a primary tumor or a metastatic lesion. In general, a selected organ contains a cell that expresses a particular target molecule such as a cell surface receptor to which an organ homing molecule can bind. By performing at least two rounds of *in vivo* panning, the selectivity of homing of the molecule to the selected organ can be determined (see Example II). As discussed above, however, in some cases an organ homing molecule can selectively home to more than one selected organ, in which case the molecule is considered to be able to selectively home to a family of selected organs.

The term "control organ" is used to mean an organ other than a selected organ. A control organ is characterized by the inability of an organ homing molecule to home to the control organ. A control organ

5 is useful for identifying non-specific binding of a molecule. A control organ can be collected, for example, to identify non-specific binding of the molecule or to determine the selectivity of homing of the molecule (see Examples I and II). In addition, non-specific binding

10 can be identified by administering, for example, a control molecule, which is known not to home to an organ but is chemically similar to a potential organ homing molecule. Alternatively, where the molecules are administered as a conjugate comprising the molecules of

15 the library linked to a support, administration of the supports, alone, can be used to identify non-specific binding. For example, a phage that expresses the gene III protein, alone, but that does not contain a peptide fusion protein, can be screened by *in vivo* panning to

20 determine the level of non-specific binding of the phage support.

In some cases it can take several rounds of *in vivo* panning to identify a control organ, since a molecule that selectively homes to an originally selected

25 organ also may have the ability to selectively home to additional organs, thus defining a family of selected organs. The ability of a molecule to home to one or to a family of selected organs allows for the preparation of panels of organ homing molecules, wherein individual

30 molecules variously home to one selected organ or to any of a family of selected organs with variable selectivity.

In general, a library of molecules, which contains a diverse population of random or selectively randomized molecules of interest, is prepared, then

35 administered to a subject. At a selected time after

administration, the subject is sacrificed and a selected
organ or part of the organ is collected such that the
molecules present in the selected organ can be
identified. For example, a mouse was injected with a
5   phage peptide display library, then, after about 1 to 4
minutes, the mouse was frozen in liquid nitrogen to
terminate circulation of the phage, the selected organ
(brain or kidney) was collected, phage present in the
selected organ were recovered and peptides that
10 selectively homed to the selected organ were identified
(see Examples I and II).

In the examples provided, the animals were
sacrificed to collect the selected organ. It should be
recognized, however, that only a part of the selected
15 organ need be collected to recover a support containing a
molecule that homes to that organ. For example, a part
of the selected organ can be collected by biopsy, such
that a molecule such as a peptide expressed by a phage,
can be administered to the same subject a second time or
20 more, as desired. Where the molecule that is to be
administered a second time to the same subject is linked,
for example, to a support, the support must be nontoxic
and should be biodegradable, so as not to interfere with
subsequent rounds of screening.

25   *In vitro* screening of phage libraries
previously has been used to identify peptides that bind
to antibodies or to cell surface receptors (Smith and
Scott, *supra*, 1993). For example, *in vitro* screening of
phage peptide display libraries has been used to identify
30 novel peptides that specifically bound to integrin
adhesion receptors (Koivunen et al., J. Cell Biol.
124:373-380 (1994a), which is incorporated herein by
reference) and to the human urokinase receptor (Goodson
et al., Proc. Natl. Acad. Sci., USA 91:7129-7133 (1994)).
35 However, such *in vitro* studies provide no insight as to

whether a peptide that can specifically bind to a
selected receptor *in vitro* also will bind the receptor *in vivo* or whether the binding peptide or the receptor are
unique to a specific organ in the body. Furthermore, the

5   *in vitro* methods are performed using defined, well-
characterized target molecules in an artificial system.
For example, Goodson et al. utilized cells expressing a
recombinant urokinase receptor. Thus, the *in vitro*
methods require prior knowledge of the target molecule

10   and yield little if any information regarding *in vivo*
utility. In contrast, the *in vivo* panning method
disclosed herein requires no prior knowledge or
availability of a target molecule. Thus, *in vivo* panning
provides a significant advantage over previous methods by

15   identifying molecules that selectively home *in vivo* to a
target molecule present in one or a few organs.

        Brain and kidney were selected as target organs
to identify phage expressing peptides that selectively
home to these selected organs because the blood vessels

20   in these organs have unique characteristics. For
example, the blood vessels in the brain form the
"blood-brain barrier" and express at least one specific
antigen (Schlosshauer and Herzog, <u>J. Cell Biol.</u> 110:1261-
1274 (1990)). Using *in vivo* panning, phage expressing

25   various peptides that selectively homed to brain or to
kidney, but not to both organs, were identified (see
Example II). Since the *in vivo* panning was terminated
about one to four minutes after intravenous
administration of the phage to the mice, the majority of

30   phage likely remained in the vasculature, indicating the
displayed peptides bound to endothelial cell surface
markers that are expressed in an organ-specific manner
(see, for example, Springer, <u>Cell</u> 76:301-314 (1994)).
Organ-specific homing of tumor cells and of lymphocytes

indicates that the vascular beds in various tissues express unique target molecules. For example, the ability of lymphocytes to return to the organ in which they were first stimulated (Salmi et al., <u>Proc. Natl. Acad. Sci., USA</u> 89:11436-11440 (1992)) and the ability of particular types of tumor cells to home or metastasize to specific organs (Fidler and Hart, <u>Science</u> 217:998-1003 (1982)) indicate that organ-specific targets are expressed in a subject.

The identification of brain homing and kidney homing peptides demonstrates that organ-specific target molecules exist and indicates that the disclosed method of *in vivo* panning can be used to identify molecules that selectively home to other organs. For example, tumor vasculature, which undergoes active angiogenesis and contains specific markers (Brooks et al., <u>Cell</u> 79:1157-1164 (1994)), is a particularly attractive target. The identification of molecules that home to tumor vasculature would provide a means for directing a therapeutic agent directly to a tumor while sparing normal tissues.

Phage peptide display libraries were constructed essentially as described Smith and Scott (*supra*, 1993; see, also, Koivunen et al., <u>Biotechnology</u> 13:265-270 (1995), which is incorporated herein by reference). Oligonucleotides encoding peptides having substantially random amino acid sequences were synthesized based on an "NNK" codon, wherein "N" is A, T, C or G and "K" is G or T. "NNK" encodes 32 triplets, which encode the twenty amino acids and an amber STOP codon (Scott and Smith, *supra*, 1990). At least one codon encoding cysteine also was included in each oligonucleotide so that cyclic peptides could be formed through disulfide linkages (see Example I). The

oligonucleotides were inserted in frame with the sequence encoding the gene III protein (gIII) in the vector fuse 5 such that a peptide-gIII fusion protein can be expressed. Following expression, the fusion protein is expressed on

5   the surface of the phage containing the vector (Smith and Scott, *supra*, 1993; Koivunen et al., *supra*, 1994b).

Remarkably, following *in vivo* panning, the phage that selectively homed to brain or to kidney displayed only a few different peptide sequences. In

10 some cases, peptides having the same amino acid sequence were encoded by phage having different oligonucleotide sequences encoding the peptide. Furthermore, a family of brain homing peptides was identified, wherein each peptide in the family contained the common amino acid

15 motif, SRL (serine-arginine-leucine), but different flanking amino acid sequences (see Table 1; SEQ ID NOS: 1, 3 and 5). In addition, two different peptides displayed the motif VLR (valine-leucine-arginine; see Table 1, SEQ ID NOS: 4 and 16). These results

20 demonstrate that it is the peptide displayed by the phage, rather than some incidental mutant property of the phage, that directs homing to the selected organ. For example, the present invention was used to identify various organ homing peptides, including brain homing

25 peptides such as CNSRLHLRC (SEQ ID NO: 1), CENWWGDVC (SEQ ID NO: 2), WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) and others as shown in Table 1, and kidney homing peptides such as CLPVASC (SEQ ID NO: 21), CGAREMC (SEQ ID NO: 22) and others as shown in Table 2.

30   The sequences of the brain and kidney homing motifs do not reveal any significant similarities with known ligands for endothelial cell receptors nor do they resemble any sequences listed in various data banks. However, some of the brain-homing motifs share a

35 similarity with integrin-binding sequences. For example,

one brain homing peptide contained an RLD sequence, which
is recognized by certain integrins (Altieri et al., J.
Biol. Chem. 265:12119-12122 (1990); Koivunen et al.,
supra, 1994a), and the DXXR (SEQ ID NO: 44) motif in

5   another peptide resembles the RGD, DGR, and NGR motifs
that bind to certain integrins (Ruoslahti, J. Clin.
Invest. 87:1-5 (1991); Koivunen et al., supra, 1994a).

      Selective homing of a molecule such as a
peptide or protein to a selected organ can be due to

10 specific recognition by the peptide of a particular cell
target molecule such as a cell surface receptor present
on a cell in the organ. Selectivity of homing is
dependent on the particular target molecule being
expressed on only one or a few different cell types, such

15 that the molecule homes to only one or a few organs. As
discussed above, brain and kidney homing peptides likely
are recognizing endothelial cell surface markers in the
blood vessels present in these organs. However, most
different cell types, particularly cell types that are

20 unique to an organ, can express unique target molecules.
Thus, in organs such as liver, spleen or lymph node,
where blood circulates through sinusoids formed by the
cells specific for the organ, in vivo panning can be
useful for identifying molecules that home to the

25 particular organ. For example, the sinusoids in liver
are formed, in part, by hepatocytes, which are unique to
liver. Using the methods disclosed herein, in vivo
panning can be used to identify molecules that
selectively home to hepatocytes and specific homing can

30 be demonstrated by performing the appropriate competition
experiments.

      An organ homing molecule such as a brain homing
(Table 1) or kidney homing (Table 2) peptide can be used
to direct a moiety to a selected organ by linking the

moiety to the molecule. As used herein, the term
"moiety" is used broadly to mean an agent linked to the
organ homing molecule. A moiety can be, for example, a
detectable label such a radiolabel; a toxin such as
5 ricin; or a drug such as a chemotherapeutic agent.
Various moieties and methods for linking a moiety to a
molecule are well known in the art and commercially
available (see, for example, Harlow and Lane, Antibodies:
A laboratory manual (Cold Spring Harbor Laboratory Press
10 1988), which is incorporated herein by reference).

          Linking of a moiety to an organ homing molecule
for the purpose of directing homing of the moiety to the
selected organ is exemplified by the linking of a brain
homing peptide to a RBC, wherein the peptide directed
15 homing of the RBC to brain (see Example II.D.). These
results indicate that an organ homing molecule of the
invention can be linked to other cell types in order to
direct the cell type to a selected organ. For example, a
tumor homing molecule identified by *in vivo* panning can
20 be linked to a white blood cell (WBC) such as a cytotoxic
T cell or a killer cell, wherein upon administration of
the tumor homing molecule/WBC complex, the molecule
directs homing of the WBC to the tumor, where the WBC can
exert its effector function.

25   An organ homing molecule also can be linked to
a moiety that is detectable external to the subject in
order to perform an *in vivo* diagnostic imaging study.
For example, *in vivo* imaging using a detectably labeled
brain homing peptide can identify a region in the brain
30 where circulation is occluded. For such studies, a gamma
ray emitting radionuclide such as indium-111 or
technitium-99 can be linked to a brain homing molecule
and, following administration to a subject, can be
detected using a solid scintillation detector.
35 Alternatively, a positron emitting radionuclide such as

carbon-11 or a paramagnetic spin label such as carbon-13 can be linked to the molecule and, following administration to a subject, the localization of the moiety/molecule can be detected using positron emission

5 transaxial tomography or magnetic resonance imaging, respectively.

Such *in vivo* imaging methods also can be used to identify the presence of cancer in a subject by linking an appropriate moiety to a tumor homing molecule,

10 which can recognize a unique target expressed by the tumor cells or by the blood vessels formed by angiogenesis in the tumor. Such methods can identify a primary tumor as well as a metastatic lesion, which may not be detectable using other methods. Having identified

15 the presence of such cancer, the tumor homing molecule can be linked to a toxin or to a chemotherapeutic agent in order to direct the moiety to the tumor. Such a method can allow selective killing of the tumor, while substantially sparing normal tissues.

20 When administered to a subject, the molecule/moiety complex is administered as a pharmaceutical composition containing, for example, the complex and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in

25 the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

A pharmaceutically acceptable carrier can

30 contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of the complex. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as

ascorbic acid or glutathione, chelating agents, low
molecular weight proteins or other stabilizers or
excipients. One skilled in the art would know that the
choice of a pharmaceutically acceptable carrier,

5   including a physiologically acceptable compound, depends,
for example, on the route of administration of the
composition. The pharmaceutical composition also can
contain an agent such as a cancer therapeutic agent.

One skilled in the art would know that a
10   pharmaceutical composition containing an organ homing
molecule can be administered to a subject by various
routes including, for example, orally or parenterally,
such as intravenously. The composition can be
administered by injection or by intubation. The
15   pharmaceutical composition also can be an organ homing
molecule linked to liposomes or other polymer matrices,
which can have incorporated therein, for example, a drug
such as a chemotherapeutic agent (Gregoriadis, Liposome
Technology, Vol. 1 (CRC Press, Boca Raton, FL 1984),
20   which is incorporated herein by reference). Liposomes,
for example, which consist of phospholipids or other
lipids, are nontoxic, physiologically acceptable and
metabolizable carriers that are relatively simple to make
and administer.

25   For the diagnostic or therapeutic methods
disclosed herein, an effective amount of the
molecule/moiety complex must be administered to the
subject. As used herein, the term "effective amount"
means the amount of the complex that produces the desired
30   effect. An effective amount often will depend on the
moiety linked to the organ homing molecule. Thus, a
lesser amount of a radiolabeled molecule can be required
for imaging as compared to the amount of a drug/molecule
complex administered for therapeutic purposes. An
35   effective amount of a particular molecule/moiety for a

specific purpose can be determined using methods well
known to those in the art.

The route of administration of an organ homing
molecule will depend, in part, on the chemical structure
5   of the molecule. Peptides, for example, are not
particularly useful when administered orally because they
can be degraded in the digestive tract. However, methods
for chemically modifying peptides to render them less
susceptible to degradation by endogenous proteases or
10   more absorbable through the alimentary tract are well
known (see, for example, Blondelle et al., *supra*, 1995;
Ecker and Crooke, *supra*, 1995; Goodman and Ro, *supra*,
1995). Such methods can be performed on peptides
identified by *in vivo* panning. In addition, methods for
15   preparing libraries of peptide analogs such as peptides
containing D-amino acids; peptidomimetics consisting of
organic molecules that mimic the structure of peptide; or
peptoids such as vinylogous peptoids, are known in the
art and can be used to identify molecules that home to a
20   selected organ and are stable for oral administration.

Organ homing molecules obtained using the
methods disclosed herein also can be useful for
identifying a target molecule such as a cell surface
receptor or a ligand for a receptor, which is recognized
25   by the organ homing peptide. For example, an organ
homing peptide can be linked to a solid support such as a
chromatography matrix. The linked peptide then can be
used for affinity chromatography by passing an
appropriately processed sample of a selected organ over
30   the column in order to bind a particular target molecule.
The bound target molecule can be eluted from the column
and can be identified and characterized by various well
known methods. In addition, an organ homing peptide can
be linked to a detectable moiety such as a radionuclide,
35   a fluorescent molecule, an enzyme or biotin and can be

used, for example, to screen a sample in order to detect the target molecule during various steps of purification of the target molecule.

The methods of the present invention were used to identify peptides that selectively home to a selected organ. For example, brain homing peptides such as CNSRLHLRC (SEQ ID NO: 1), CENWWGDVC (SEQ ID NO: 2), and others as shown in Table 1 were identified. In addition, brain homing peptides comprising the motif, SRL (serine-arginine-lysine), such as the peptide CLSSRLDAC (SEQ ID NO: 3), or the motif VLR, such as the peptide WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16), were identified. Kidney homing peptides such as CLPVASC (SEQ ID NO: 21), CGAREMC (SEQ ID NO: 22) and other as shown in Table 2 also were identified.

Cysteine residues were included in the peptides such that cyclization of the peptides could be effected. However, in some cases one or both of the cysteine residues in a peptide can be deleted without significantly affecting the organ homing activity of a peptide of the invention. Thus, a peptide having the sequence LSSRLDA (SEQ ID NO: 19; compare SEQ ID NO: 3) also can be a brain homing peptide. Similarly, the amino acid residues N-terminal and C-terminal to the first and last cysteine residues, respectively, in a peptide such as WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) can be dispensable without substantially altering brain homing activity of the peptide. Thus, a peptide having the sequence VLREGPAGG (SEQ ID NO: 20) also can be useful as a brain homing peptide. Methods for determining the necessity of a cysteine residue or of amino acid residues N-terminal or C-terminal to a cysteine residue for organ homing activity of a peptide of the invention are routine and well known in the art.

An organ homing peptide is useful, for example, for targeting a desired moiety to the selected organ as discussed above. In addition, an organ homing peptide can be used to identify the presence of a target molecule in a sample. As used herein, the term "sample" is used in its broadest sense to mean a cell, tissue, organ or portion thereof that is isolated from the body. A sample can be, for example, a histologic section or a specimen obtained by biopsy or cells that are placed in or adapted to tissue culture. If desired, a sample can be processed, for example, by homogenization, which can be an initial step for isolating the target molecule to which an organ homing molecule binds.

An organ homing peptide such as a brain homing peptide can be used to identify the target molecule expressed in brain. For example, a brain homing peptide can be attached to a matrix such as a chromatography matrix to produce a peptide affinity matrix. A homogenized sample of brain can be applied to the peptide-affinity matrix under conditions that allow specific binding of the brain homing peptide to the target molecule (see, for example, Deutshcer, Meth. Enzymol., Guide to Protein Purification (Academic Press, Inc., ed. M.P. Deutscher, 1990), Vol. 182, which is incorporated herein by reference; see, for example, pages 357-379). Unbound and non-specifically bound material can be removed and the specifically bound brain-derived target molecule can be isolated in substantially purified form.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

IN VIVO PANNING

This example demonstrates methods for preparing a phage library and screening the library using *in vivo*
5   panning to identify phage expressing peptides that home to a selected organ.

A.   Preparation of phage libraries:

Phage display libraries were constructed using the fuse 5 vector as described by Koivunen et al. (*supra*,
10   1995; see, also, Koivunen et al. Meth. Enzymol. 245:346-369 (1994b), which is incorporated herein by reference). Six libraries encoding peptides designated CX_5C (SEQ ID NO: 36), CX_6C (SEQ ID NO: 37), CX_7C (SEQ ID NO: 38), CX_9 (SEQ ID NO: 39), $X_2CX_{14}CX_2$ (SEQ ID NO: 40) and
15   X_2CX_{18} (SEQ ID NO: 41) were prepared, where "C" indicates cysteine and "X_N" indicates the given number of individually selected amino acids. These libraries can display cyclic peptides when at least two cysteine residues are present in the peptide.

20   Oligonucleotides were constructed such that "C" was encoded by the codon TGT and "X_N" was encoded by NNK, where "N" is equal molar mixtures of A, C, G and T, and where "K" is equal molar mixtures of G and T. Thus, the peptide represented by CX_5C (SEQ ID NO: 36) can be
25   represented by an oligonucleotide having the sequence TGT(NNK)$_5$TGT (SEQ ID NO: 42). Oligonucleotides were made double stranded by 3 cycles of PCR amplification, purified and ligated to the nucleic acid encoding the gene III protein in the fuse 5 vector such that, upon
30   expression, the peptide is present as a fusion protein at the N-terminus of the gene III protein.

The vectors were transfected by electroporation into MC1061 cells. Bacteria were cultured for 24 hr in the presence of 20 μg/ml tetracycline, then phage were collected from the supernatant by precipitation twice

5 using polyethylene glycol. Each library contained about 5 x 10^9 t0 5 x 10^{14} transducing units (TU; individual recombinant phage).

B. In vivo panning of phage:

A mixture of phage libraries containing 1 x 10^{14}

10 TU was diluted in 200 μl DMEM and injected into the tail vein of anesthetized BALB\c mice (2 month old females; Jackson Laboratories; Bar Harbor ME); Avertin (0.015 ml/g) was used as anesthetic. After 1-4 minutes, mice were snap frozen in liquid nitrogen. To recover the

15 phage, carcasses were partially thawed at room temperature for 1 hr, organs were collected and weighed, then were ground in 1 ml DMEM-PI (DMEM containing protease inhibitors (PI); phenylmethylsulfonyl fluoride (PMSF; 1 mM), aprotinin (20 μg/ml), leupeptin (1 μg/ml)).

20 Organ samples were washed 3 times with ice cold DMEM-PI containing 1% bovine serum albumin (BSA), then directly incubated with 1 ml K91-kan bacteria for 1 hr. Ten ml NZY medium containing 0.2 μg/ml tetracycline (NZY/tet) was added to the bacterial culture, the mixture

25 was incubated in a 37°C shaker for 1 hr, then 200 μl aliquots were plated in agar plates containing 40 μg/ml tetracycline (tet/agar).

Individual colonies containing phage recovered from brain or kidney were grown for 16 hr in 5 ml

30 NZY/tet. The bacterial cultures obtained from the individual colonies were pooled and the amplified eluates were injected into mice as described above for a second round of in vivo panning. A third round of panning also

was performed. Phage DNA was purified from individual bacterial colonies obtained from the second and the third round of *in vivo* panning and the DNA sequences encoding the peptides expressed by selected phage were determined

5   (see Koivunen et al., *supra*, 1994b).

EXAMPLE II

<u>CHARACTERIZATION OF PEPTIDES THAT HOME
TO A SELECTED ORGAN</u>

This example demonstrates that an organ homing
10   peptide of the invention selectively homes to a selected organ and that an organ homing peptide identified by *in vivo* panning can be used to direct a moiety to a selected organ.

<u>A.   Brain is the selected organ</u>

15   Three rounds of *in vivo* panning in mice were

performed. Kidney was used as a control organ. Mice were injected with two different mixtures of phage libraries. The first mixture contained libraries encoding CX_9 (SEQ ID NO: 39), CX_5C (SEQ ID NO: 36), CX_6C
20   (SEQ ID NO: 37) and CX_7C (SEQ ID NO: 38) peptides (CX_{5-7}/CX_9 mixture; SEQ ID NOS: 36-39). The second mixture contained libraries encoding $X_2CX_{14}CX_2$ (SEQ ID NO: 40) and X_2CX_{18} (SEQ ID NO: 41) peptides ($X_2CX_{18}/X_2CX_{14}CX_2$ mixture; SEQ ID NOS: 40 and 41).

25   The phage library mixtures were administered to mice via tail vein injection. Phage input was 1 x 10^{16} TU of the CX_{5-7}/CX_9 (SEQ ID NOS: 36-39) mixture or 1 x 10^{14} TU of the $X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) mixture. Phage were recovered from the brains of the injected
30   mice, the recovered phage were amplified *in vitro*, then a second and a third round of *in vivo* panning was

performed. During the second and third rounds of panning, phage were recovered from brain and from kidney and the number of TU from each organ was compared. This comparison revealed that 6x more phage from the CX_{5-7}/CX_9

5 (SEQ ID NOS: 36-39) mixture bound to brain than to kidney in the second round and 13x more of the CX_{5-7}/CX_9 (SEQ ID NOS: 36-39) phage bound to brain than to kidney in the third round of panning. Following administration of the $X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) mixture, an 11x and

10 8x enrichment of phage homing to the brain as compared to kidney occurred during the second and third rounds of panning, respectively. Thus, substantial enrichment of phage binding to the brain was observed following the second and third rounds of *in vivo* panning.

15   The amino acid sequences were determined for the inserts present in 73 cloned phage that were recovered from brain during the second and third rounds of *in vivo* panning. Peptides containing an SRL motif predominated (36% of the clones sequenced; see SEQ ID

20 NOS: 1, 3 and 5), followed by peptides containing the VLR motif (20.5% of the clones; see SEQ ID NOS: 4 and 16) and the peptide CENWWGDVC (SEQ ID NO: 2; 19% of the clones; see Table 1). Other peptides that occurred much less frequently, but were present more than once, included

25 CGVRLGC (SEQ ID NO: 6), CKDWGRIC (SEQ ID NO: 7), CLDWGRIC (SEQ ID NO: 8) and CTRITESC (SEQ ID NO: 9). Nine other sequences appeared only one time each and were not characterized further.

TABLE 1

PEPTIDES FROM PHAGE RECOVERED FROM BRAIN

A.A. SEQUENCE (SEQ ID NO:)	# PHAGE DISPLAYING SAME A.A. SEQUENCE (% PHAGE)
$CX_{5-7}C/CX_9$ (36-39) library:	
CNSRLHLRC (1)	16 (21.9%)
CENWWGDVC (2)	14 (19.2%)
CLSSRLDAC (3)	6 (8.2%)
CVLRGGRC (4)	5 (6.8%)
CNSRLQLRC (5)	4 (5.5%)
CGVRLGC (6)	3 (4.1%)
CKDWGRIC (7)	2 (2.8%)
CLDWGRIC (8)	2 (2.8%)
CTRITESC (9)	2 (2.8%)
CETLPAC (10)	1 (1.4%)
CRTGTLFC (11)	1 (1.4%)
CGRSLDAC (12)	1 (1.4%)
CRHWFDVVC (13)	1 (1.4%)
CANAQSHC (14)	1 (1.4%)
CGNPSYRC (15)	1 (1.4%)
$X_2CX_{18}/X_2CX_{14}CX_2$ (SEQ ID NOS: 40 and 41) library:	
WRCVLREGPAGGCAWFNRHRL (16)	10 (13.7%)
YPCGGEAVAGVSSVRTMCSE (17)	1 (1.4%)
LNCDYQGTNPATSVSVPCTV (18)	1 (1.4%)

The SRL tripeptide motif was present in several different sequence contexts, indicating that the nucleic acids encoding the peptides were derived from a number of independent phage. These results indicate that the

5　selection of the peptides containing the SRL motif represents the specific binding of several independent phage displaying peptides with the SRL sequence and is not an artifact due, for example, to phage amplification. In addition, in some cases, different phage expressed

10　peptides that had the same amino acid sequence, but were encoded by oligonucleotides having different sequences, thus confirming that homing of a particular phage to an organ is due to the specific peptide expressed on the phage.

15　　　　To determine the specificity of brain homing of the individual motifs identified, phage displaying the predominant motifs were amplified individually, diluted to the same input titer and administered to mice. Following administration, brain and kidney were removed

20　and the number of TU of phage in each organ was determined. The enrichment ratio of phage recovered from the selected organ, brain, as compared to the control organ, kidney, revealed that phage displaying one of the four most recovered peptides, CNSRLHLRC (SEQ ID NO: 1),

25　CENWWGDVC (SEQ ID NO: 2), WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16), CLSSRLDAC (SEQ ID NO: 3), each selectively targeted the brain as compared to the kidney. Specifically, the ratio of selective homing (brain:kidney) was about 8 for CNSRLHLRC (SEQ ID NO: 1)

30　and for CLSSRLDAC (SEQ ID NO: 3), 4 for CENWWGDVC (SEQ ID NO: 2) and 9 for WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16). Two control phage showed low binding to both brain and kidney. These results demonstrate that *in vivo* panning can be used to screen phage display libraries in order to

identify phage expressing peptides that home to a
selected organ.

B. Kidney as the selected organ

The same methodology used to isolate phage
5   expressing brain homing peptides was used to isolate
phage expressing peptides that home to kidney. In these
experiments, brain was used as the control organ. A
mixture of the CX_5C (SEQ ID NO: 36) and the CX_6C (SEQ ID
NO: 37) libraries was administered as described above.
10 Homing of phage to the kidney was obtained and an
approximately 3x to 7x enrichment of phage homing to
kidney was observed following a second round of *in vivo*
panning.

The amino acid sequences were determined for
15 the inserts in 48 cloned phage that homed to kidney. The
peptides expressed by these phage were represented by two
predominant sequences, CLPVASC (SEQ ID NO: 21; 46% of the
clones sequenced) and CGAREMC (SEQ ID NO: 22; 17% of the
clones; see Table 2). In addition, the peptide CKGRSSAC
20 (SEQ ID NO: 23) appeared three times and three other
peptides were present twice each. Phage expressing a
CLPVASC (SEQ ID NO: 21), CGAREMC (SEQ ID NO: 22) or
CKGRSSAC (SEQ ID NO: 23) peptide each exhibited selective
homing to kidney; the ratio of selective kidney:brain
25 homing was 7 for CLPVASC (SEQ ID NO: 21), 3 for CGAREMC
(SEQ ID NO: 22) and 2 for CKGRSSAC (SEQ ID NO: 23).

These results demonstrate that the *in vivo*
panning method is a generally applicable method for
screening a phage library to identify phage expressing
30 peptides that home to a selected organ. Database
searches did not reveal any significant homology of the

TABLE 2

PEPTIDES FROM PHAGE RECOVERED FROM KIDNEY

A.A. SEQUENCE (SEQ ID NO:)		# PHAGE DISPLAYING SAME A.A. SEQUENCE (% PHAGE)
CLPVASC	(21)	22 (45.8%)
CGAREMC	(22)	8 (16.7%)
CKGRSSAC	(23)	3 (6.2%)
CWARAQGC	(24)	2 (4.2%)
CLGRSSVC	(25)	2 (4.2%)
CTSPGGSC	(26)	2 (4.2%)
CMGRWRLC	(27)	1 (2.1%)
CVGECGGC	(28)	1 (2.1%)
CVAWLNC	(29)	1 (2.1%)
CRRFQDC	(30)	1 (2.1%)
CLMGVHC	(31)	1 (2.1%)
CKLLSGVC	(32)	1 (2.1%)
CFVGHDLC	(33)	1 (2.1%)
CRCLNVC	(34)	1 (2.1%)
CKLMGEC	(35)	1 (2.1%)

brain homing or kidney homing peptides to known ligands for endothelial cell receptors.

C. Peptide homing is specific:

In order to confirm the specificity of a peptide for directing homing to a selected organ, peptide competition experiments were performed. The cyclic peptide, CLSSRLDAC (SEQ ID NO: 3), which is one of the

brain homing peptides (see Table 1) was synthesized (Immunodynamics; La Jolla CA) and purified by HPLC. The effect on the homing of phage expressing CLSSRLDAC (SEQ ID NO: 3), CENWWGDVC (SEQ ID NO: 2) or

5   WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) to the brain was examined in order to determine whether co-administration of the synthetic peptide affected homing of the phage.

Phage expressing CLSSRLDAC (SEQ ID NO: 3), CENWWGDVC (SEQ ID NO: 2) or WRCVLREGPAGGCAWFNRHRL (SEQ ID

10   NO: 16) were titrated to the same concentration and 1×10^{8} TU was injected into mice alone, or with 100 μg purified synthetic cyclic CLSSRLDAC (SEQ ID NO: 3) peptide. The synthetic CLSSRLDAC (SEQ ID NO: 3) peptide inhibited the homing of phage expressing CLSSRLDAC (SEQ

15   ID NO: 3) by about 60%. This result demonstrates that the homing of the phage to brain is specifically due to the expression on the phage of the CLSSRLDAC (SEQ ID NO: 3) peptide.

The synthetic CLSSRLDAC (SEQ ID NO: 3) peptide

20   also inhibited homing of phage expressing the WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16) peptide by about 60%, but did not affect homing of CENWWGDVC (SEQ ID NO: 2) phage. This result indicates that the CLSSRLDAC (SEQ ID NO: 3) and WRCVLREGPAGGCAWFNRHRL (SEQ ID NO: 16)

25   peptides can recognize the same target molecule in brain, whereas the CENWWGDVC (SEQ ID NO: 2) peptide recognizes a different target.

D.   The brain homing peptide CLSSRLDAC (SEQ ID NO: 3)
     directs red blood cells to brain

30

The synthetic cyclic CLSSRLDAC (SEQ ID NO: 3) peptide (1 mg) was labeled with iodine-125 using the Bolton and Hunter reagent (Amersham; Arlington Heights IL). Labeled peptide was purified by reverse phase HPLC

on Sep-Pak™ cartridges (Waters, Millipore Corp.; Milford PA) and the ^{125}I-peptide (200 μg) was coupled to 1 ml glutaraldehyde-stabilized sheep RBC (Sigma Chemical Co.; St. Louis MO) according to the manufacturer's

5   instructions.

Fifty μl ^{125}I-peptide/RBC (200,000 cpm), alone or with 10 mM of unlabeled CLSSRLDAC (SEQ ID NO: 3), which is a brain homing peptide, or CVRLNSLAC (SEQ ID NO: 43), which has no brain homing activity, was injected

10   into the tail vein. After 2 min, each mouse was perfused through the heart with 50 ml DMEM and the brain and kidney were removed and assayed for radioactivity.

Approximately twice as much of the CLSSRLDAC/RBC complex (SEQ ID NO: 3) homed to brain than

15   to kidney. Coadministration of unlabeled CLSSRLDAC (SEQ ID NO: 3) with the complex essentially completely inhibited brain homing of the complex but had no effect on complex localizing to the kidney, indicating that localization of the complex in the kidney was

20   non-specific. Coadministration of unlabeled CVRLNSLAC (SEQ ID NO: 43) had no effect on selective homing of the complex to brain and no effect on the non-specific localization of the complex in the kidney. These results demonstrate that an organ homing peptide identified using

25   *in vivo* panning can be linked to a moiety such as a blood cell and selectively directs homing of the linked moiety to the selected organ.

Although the invention has been described with reference to the disclosed examples, it should be

30   understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Ruoslahti, Erkki
                  Pasqualini, Renata

   (ii) TITLE OF INVENTION: Method of Identifying Molecules That
        Home to a Selected Organ In Vivo

  (iii) NUMBER OF SEQUENCES: 44

   (iv) CORRESPONDENCE ADDRESS:
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    (v) COMPUTER READABLE FORM:
       (A) MEDIUM TYPE: Floppy disk
       (B) COMPUTER: IBM PC compatible
       (C) OPERATING SYSTEM: PC-DOS/MS-DOS
       (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

   (vi) CURRENT APPLICATION DATA:
       (A) APPLICATION NUMBER:
       (B) FILING DATE:
       (C) CLASSIFICATION:

  (vii) PRIOR APPLICATION DATA:
       (A) APPLICATION NUMBER: US 08/526,710
       (B) FILING DATE: 11-SEP-1995

  (vii) PRIOR APPLICATION DATA:
       (A) APPLICATION NUMBER: US 08/813,273
       (B) FILING DATE: 10-MAR-1997

  (vii) PRIOR APPLICATION DATA:
       (A) APPLICATION NUMBER: US 08/862,855
       (B) FILING DATE: 23-MAY-1997

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Asn Ser Arg Leu His Leu Arg Cys
1   5

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Glu Asn Trp Trp Gly Asp Val Cys
1   5

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Leu Ser Ser Arg Leu Asp Ala Cys
1   5

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Cys Val Leu Arg Gly Gly Arg Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Cys Asn Ser Arg Leu Gln Leu Arg Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Cys Gly Val Arg Leu Gly Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Cys Lys Asp Trp Gly Arg Ile Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Leu Asp Trp Gly Arg Ile Cys
1   5

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Thr Arg Ile Thr Glu Ser Cys
1   5

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Glu Thr Leu Pro Ala Cys
1   5

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Thr Gly Thr Leu Phe Cys
1   5

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 8 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Gly Arg Ser Leu Asp Ala Cys
1   5

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 9 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Arg His Trp Phe Asp Val Val Cys
1   5

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 8 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Ala Asn Ala Gln Ser His Cys
1   5

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

    Cys Gly Asn Pro Ser Tyr Arg Cys
    1   5

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

    Trp Arg Cys Val Leu Arg Glu Gly Pro Ala Gly Gly Cys Ala Trp Phe
    1   5   10   15

    Asn Arg His Arg Leu
            20

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

    Tyr Pro Cys Gly Gly Glu Ala Val Ala Gly Val Ser Ser Val Arg Thr
    1   5   10   15

    Met Cys Ser Glu
            20

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Asn Cys Asp Tyr Gln Gly Thr Asn Pro Ala Thr Ser Val Ser Val
1   5   10   15

Pro Cys Thr Val
      20

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Ser Ser Arg Leu Asp Ala
1   5

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Val Leu Arg Glu Gly Pro Ala Gly Gly
1   5

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Leu Pro Val Ala Ser Cys
1   5

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Cys Gly Ala Arg Glu Met Cys
1   5

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Cys Lys Gly Arg Ser Ser Ala Cys
1   5

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Cys Trp Ala Arg Ala Gln Gly Cys
1   5

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

   Cys Leu Gly Arg Ser Ser Val Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

   Cys Thr Ser Pro Gly Gly Ser Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

   Cys Met Gly Arg Trp Arg Leu Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Val Gly Glu Cys Gly Gly Cys
1   5

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Val Ala Trp Leu Asn Cys
1   5

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Arg Arg Phe Gln Asp Cys
1   5

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Leu Met Gly Val His Cys
1   5

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

   Cys Lys Leu Leu Ser Gly Val Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:33:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

   Cys Phe Val Gly His Asp Leu Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

   Cys Arg Cys Leu Asn Val Cys
   1   5

(2) INFORMATION FOR SEQ ID NO:35:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Cys Lys Leu Met Gly Glu Cys
1   5

(2) INFORMATION FOR SEQ ID NO:36:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

    Cys Xaa Xaa Xaa Xaa Xaa Cys
    1   5

(2) INFORMATION FOR SEQ ID NO:37:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

    Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
    1   5

(2) INFORMATION FOR SEQ ID NO:38:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

    Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    1   5

(2) INFORMATION FOR SEQ ID NO:39:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:40:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                      15

Xaa Cys Xaa Xaa
            20
```

(2) INFORMATION FOR SEQ ID NO:41:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                      15

Xaa Xaa Xaa Xaa Xaa
            20
```

(2) INFORMATION FOR SEQ ID NO:42:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TGTNNKNNKN NKNNKNNKTG T   21

(2) INFORMATION FOR SEQ ID NO:43:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Cys Val Arg Leu Asn Ser Leu Ala Cys
1   5

(2) INFORMATION FOR SEQ ID NO:44:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 4 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Xaa Xaa Arg
1